Supplementary materials:

The PI3K/AKT/mTOR signaling regulates BCP ceramic-induced osteogenesis

Peijie Tan[1], Yuchen Hua[1], Bo Yuan[1], Xiaoyang Liu[3], Xuening Chen[1], Wei-Nan Zeng[3], Qin Zeng[1, 2, *], Xiangdong Zhu[1, *], Xingdong Zhang[1,2]

[1]National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, China, 610064

[2]NMPA Key Laboratory for Quality Research and Control of Tissue Regenerative Biomaterials & Institute of Regulatory Science for Medical Devices & NMPA Research Base of Regulatory Science for Medical Devices, Sichuan University, Chengdu, China, 610064

[3]Orthopedic Research Institution, Department of Orthopedics, West China Hospital, Sichuan University, Chengdu 610041, China

*Corresponding authors: E-mail: qzeng8156@scu.edu.cn; zhu_xd1973@scu.edu.cn

NO.29 Wangjiang Road, Chengdu, China, 610064

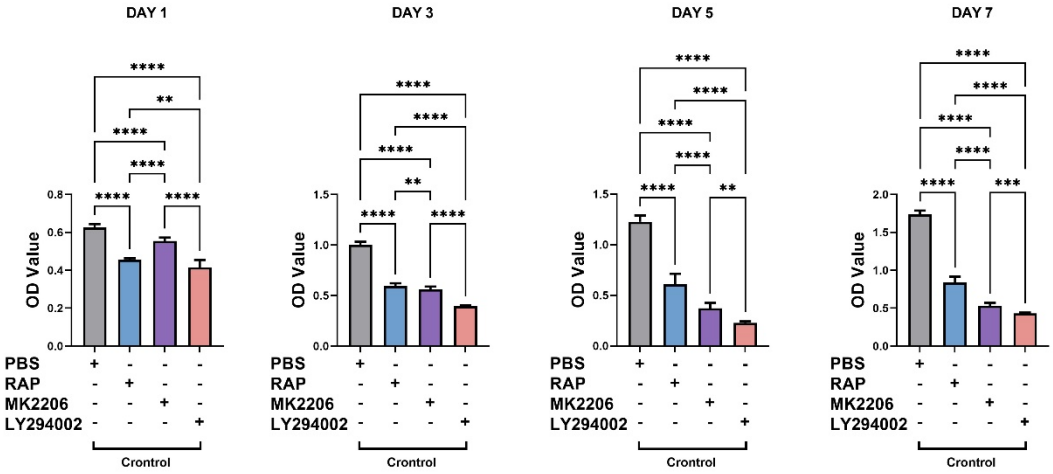


Fig.s1. The OD value of CCK-8 revealing the proliferation activity of BMSCs with or without the supplement of RAP, MK2206, or LY294002 to block mTOR, AKT or PI3K signaling of the cells at day 1, day 3, day 5, day 7. One-way ANOVA with LSD post hoc test for multiple comparisons was performed for statistical analysis with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

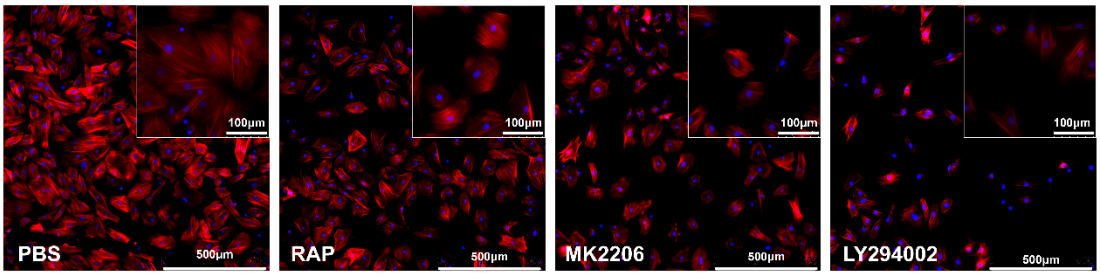


Fig.s2. Observation of actin cytoskeleton after BMSCs was treated with PI3K, AKT or mTOR signal inhibitor for 3 days.

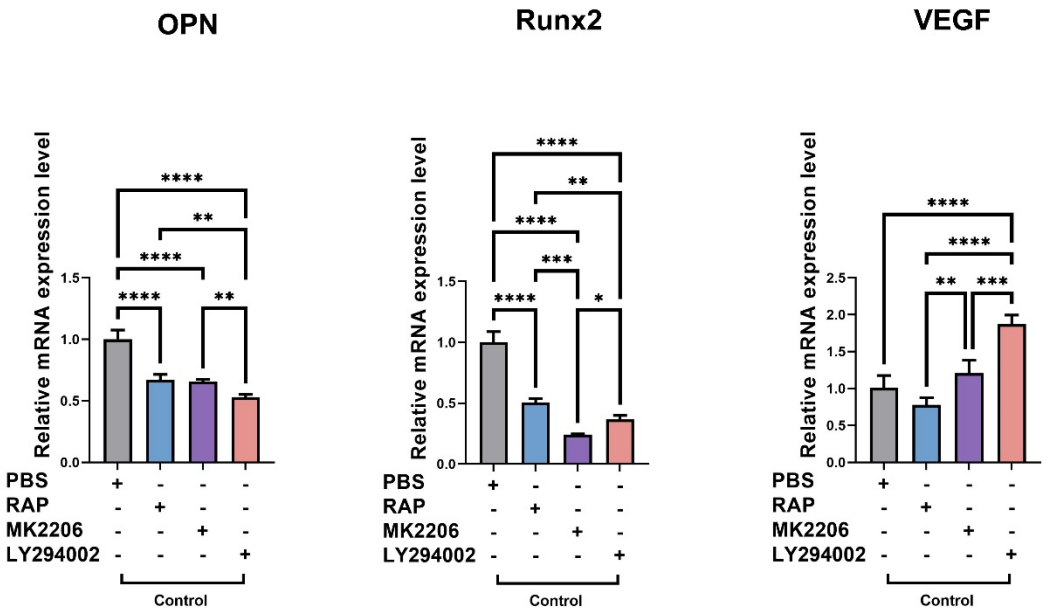
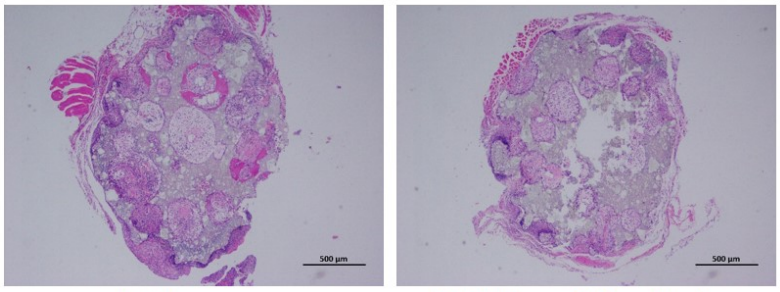


Fig.s3. Inhibiting the activation of PI3K, AKT, or mTOR signaling suppressed the expression of osteogenic gene OPN (A), RUNX2(B), and pro-angiogenic gene VEGF (C) in BMSCs. One-way ANOVA with LSD post hoc test for multiple comparisons was performed for statistical analysis with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

A

Materials Dose of Rap	BCP (0)		BCP+Rap (0.02mg/kg)		BCP+Rap (0.2mg/kg)	BCP+Rap (2mg/kg)	
#Osteogenic specimen/#Total	3/4			1/4	0/4	1/4	
	(3/4)	(1/4)	(1/4)	(3/4)	(4/4)	(1/4)	(3/4)
Bone area (%)	2±1.36	0	1.41	0	0	0.51	0

B



BCP BCP+Rap (0.2mg/kg)

Fig.s4. Administration of RAP at different doses prevented the new bone formation in the BCP ceramic implants at week 6. The number of osteogenic specimens among the total number of specimens and proportion of new bone in the BCP ceramic implants with or without additional treatment of RAP at different doses (**A**). The representative H&E image of sections of BCP ceramics with or without additional treatment of RAP at 0.2 mg/kg (**B**).

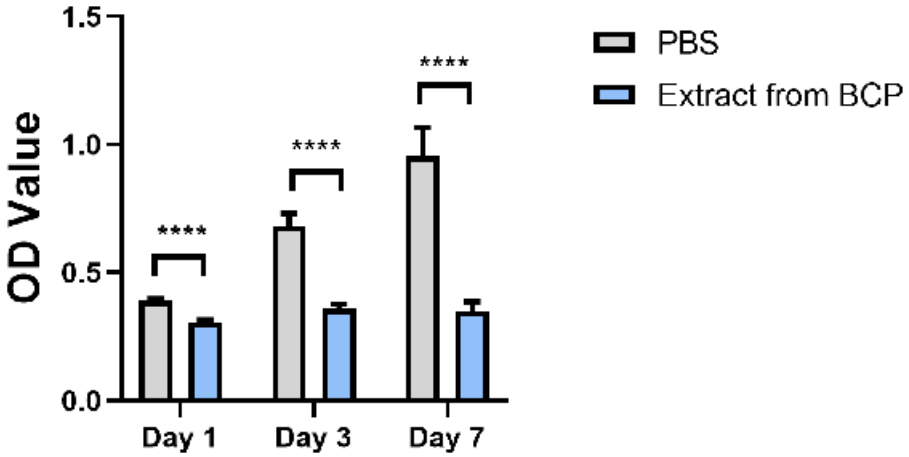


Fig.s5. The extract from BCP ceramics suppressed the proliferation of BMSCs compared to the control group. The preparation method of BCP extract was referred to ISO 10993-12 2007. A BCP ceramic (Φ14 × 2 mm) was soaked in 1mL of culture medium in the 24-well plates at 37 ℃ for 72 hours, and then the extract was collected for the cell proliferation assay. The cell proliferation was determined by CCK-8. The One-way ANOVA with LSD post hoc test for multiple comparisons was performed for statistical analysis with ****$p < 0.0001$.

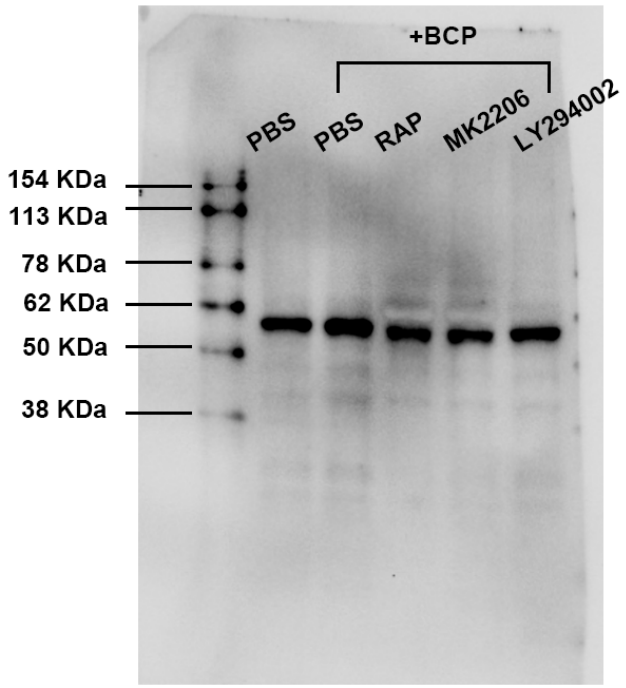


Fig s6. The original scanned image of the western blot for AKT in Figure 3A

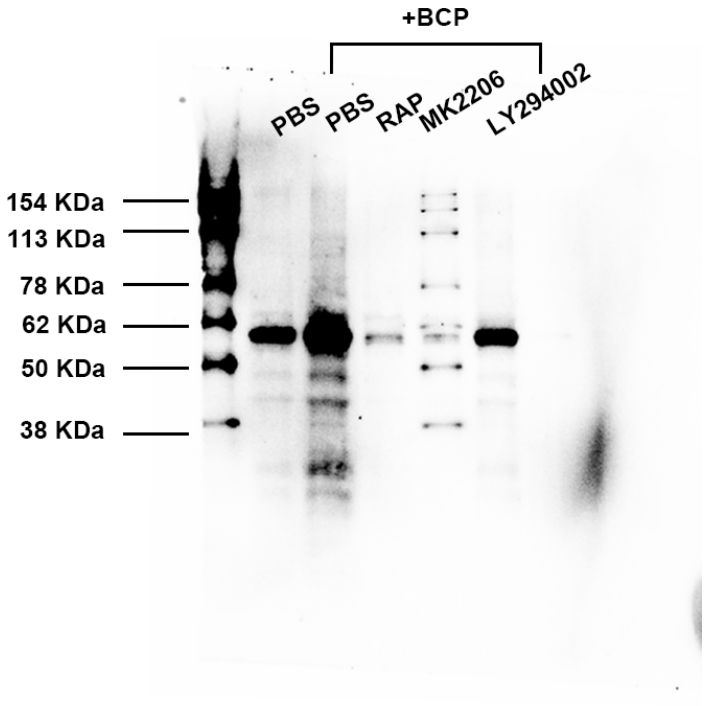


Fig s7. The original scanned image of the western blot for P-AKT in Figure 3A**.**

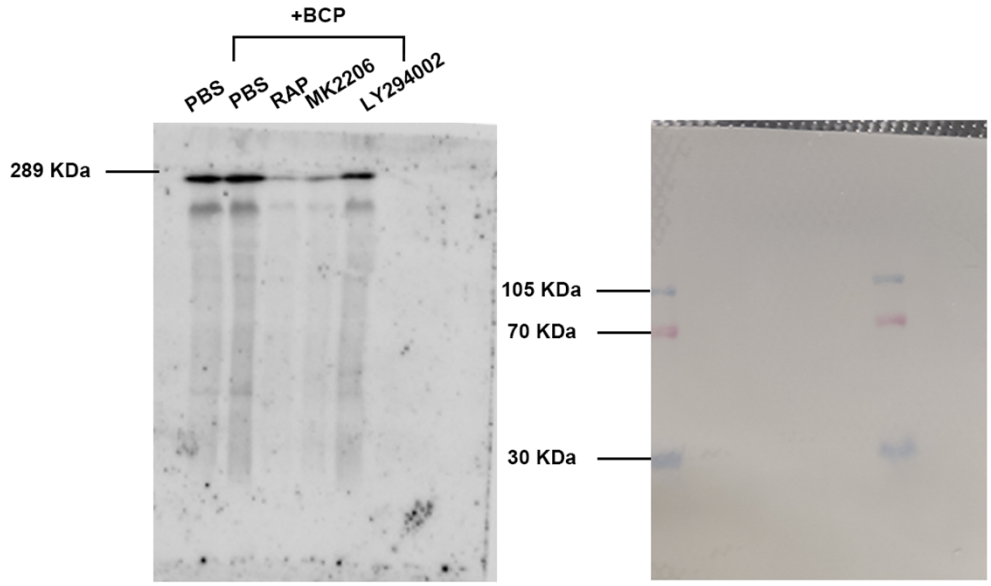


Fig s8. The original scanned image of the western blot for mTOR in Figure 3A**.**

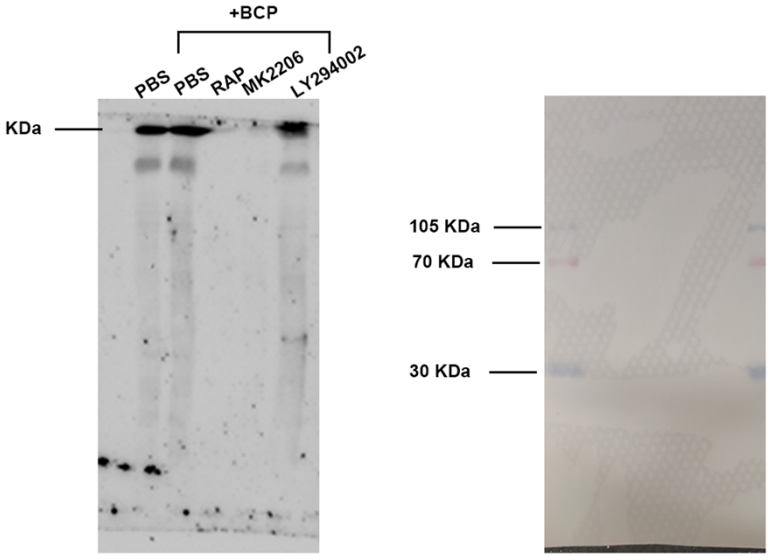


Fig s9. The original scanned image of the western blot for P-mTOR in Figure 3A**.**

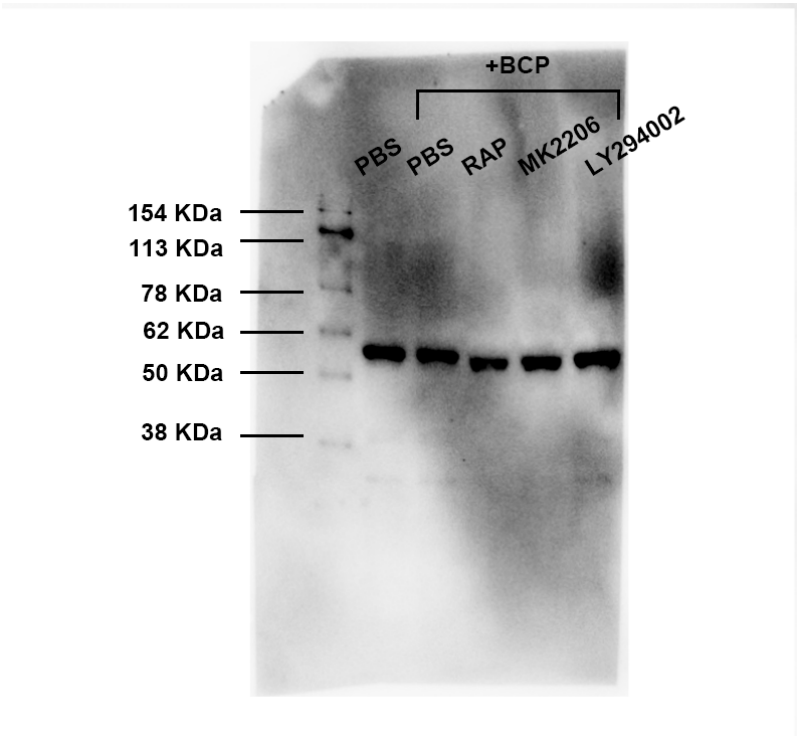


Fig s10. The original scanned image of the western blot for AKT in Figure 5B**.**

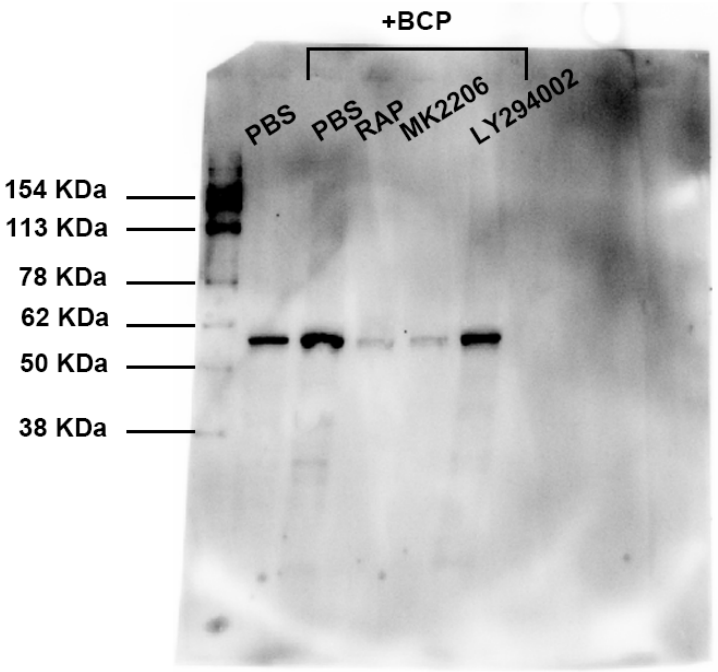


Fig s11. The original scanned image of the western blot for P-AKT in Figure 5B**.**

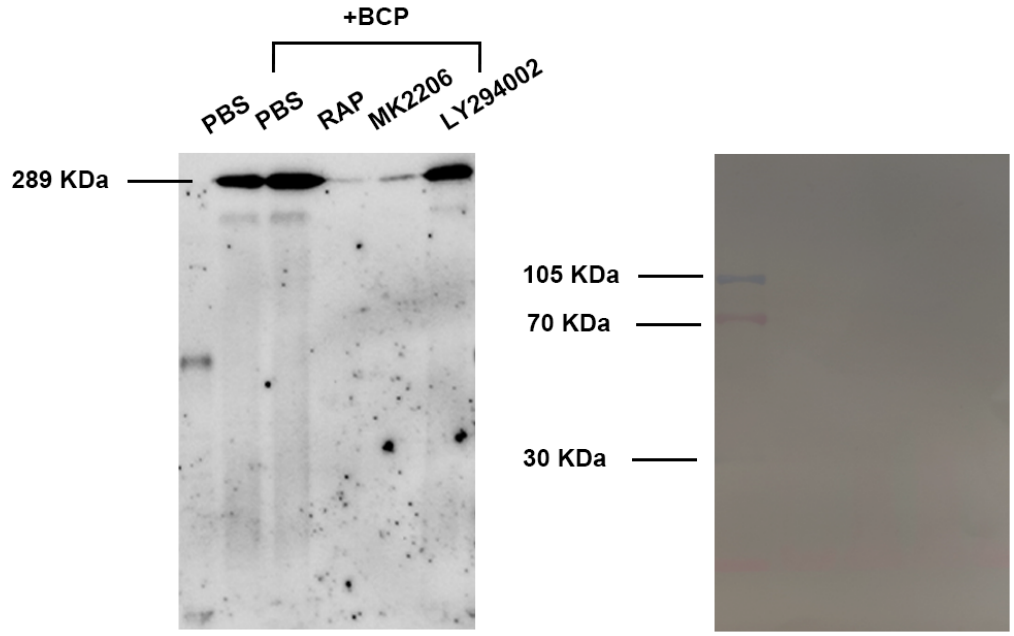


Fig s12. The original scanned image of the western blot for mTOR in Figure 5B**.**

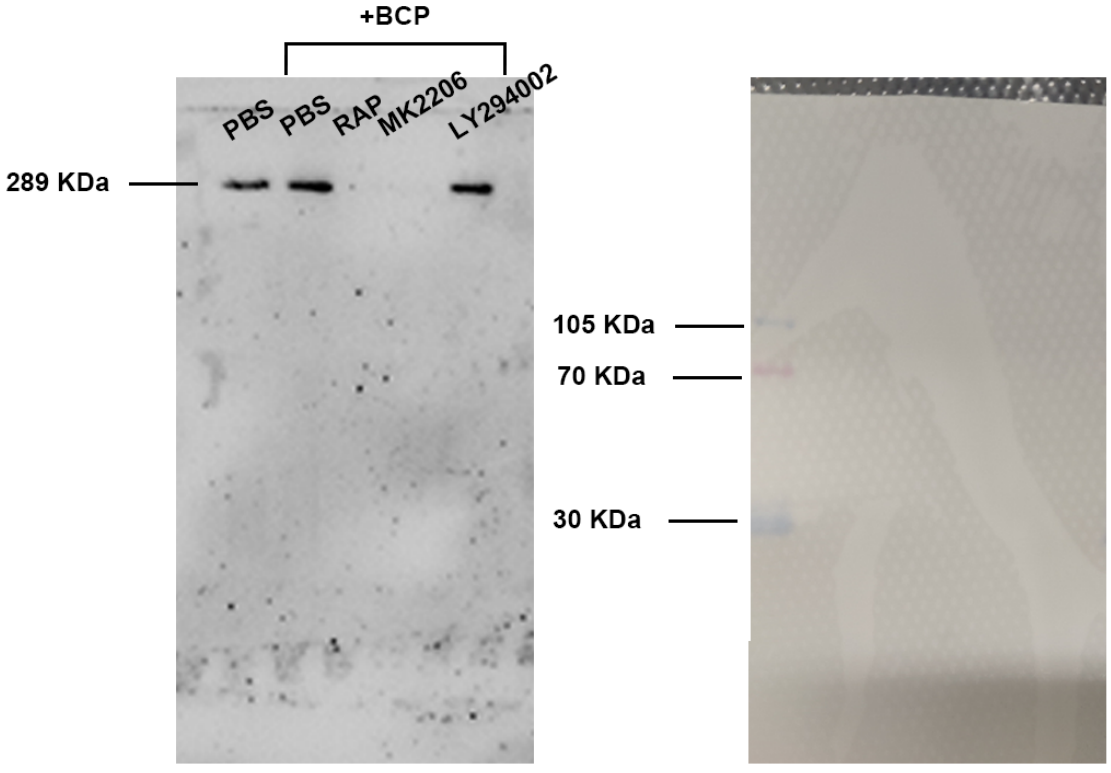


Fig s13. The original scanned image of the western blot for P-mTOR in Figure 5B**.**